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Fermentation process and starter culture

Field of the invention

The present invention relates to the field of biotechnology, and in particular to ethanol production through the fermentation of one or more organic starting materials. Specifically, the invention relates to a process for ethanol production wherein a fungus capable of metabolizing 5-carbon compounds is used to produce ethanol and/or to enhance the ethanol yield.

Background of the invention

The use of fossil fuels has contributed to environmental problems, including the increased emission of CO_2, a gas implicated in global warming. A significant increase in atmospheric CO_2 concentration has been recorded during the past 350 years. The use of renewable resources as an alternative to fossil fuels has been under investigation for many years. Compared to the increasing use of fossil fuels, which is a limited resource, the production of ethanol from biomass offers a promising alternative.

Ethanol can be regarded as a more environmental friendly fuel than fossil fuels because it adds only little net CO_2 to the atmosphere. Considerable research efforts are therefore conducted to find economical ways of producing ethanol from renewable raw materials. Ethanol from biomass is produced through fermentation of sugar-containing materials. The biomass of interest can be feed stocks such as sugarcane or maize, but the raw material cost would then be approximately 40-70% of the total ethanol cost. Therefore low cost raw material such a lignocellulosic materials e.g. fast growing trees, grass, waste products such as agricultural and forestry residues, for the production of ethanol are of great commercial and environmental interest to make ethanol competitive with fossil fuels. The use of lignocellulosic materials is apparently very advantageous, because it is the most abundant renewable organic material in the biosphere.

The primary components of most plant material are commonly described as lignocellulosic biomass. Lignocellulose is composed of three major constituents i.e. cellulose (35-50%), hemi-cellulose (20–35%) and lignin. Minor constituents of lignocellulose are ash, phenolics, extractives and trace residues. The major compound cellulose is a linear polymer of D-glucose linked together by β-1,4-

glucosodic bonds to create a water-insoluble, crystalline material. The cellulose molecules are organized in elementary fibrils associated with hydrogen and van der Waals bonds, forming a very rigid structure of microfibrils. The microfibrils contains regions of amorphous structure that are susceptible to hydrolysis. The second most frequent compound, hemicellulose, is a branched polymer of different monomeric sugars. Hemicellulose links through hydrogen bonds to cellulose and through covalent bonds to lignin. The third most frequent compound in wood is lignin, which is one of the most abundant substances in the plant world. Lignin significantly increases the mechanical strength of the wood. Relatively few microorganisms can utilize lignin effectively, which makes wood a very durable material.

The production of ethanol from fermentation of sugars in biomass is of large economic and environmental interest. Cellulose, lignocellulose and hemicellulose in the biomass all consist of long chains of sugar molecules. In order to get the ethanol production working, the sugar molecules needs to be separated in a process called hydrolysis from the long chains where they are stored. For example, the most common way of degrading lignocellulose in the pulp and paper industry is the mechanical-chemical way. The wood material is mechanically decomposed into smaller fractions in combination with an acid. The sugars can then be converted to ethanol using appropriately selected microorganisms in a process called fermentation. Fermentation is the anaerobic breakdown of organic compounds by living cells, often with the production of heat and waste gases and a wide variety of end-products (e.g. ethanol).

Prior art

The production of ethanol from lignocellulose is a process, which can be optimized in a variety of ways. Ethanol production from industrial lignocellulose material has been the focus of considerable research. Most reports are dealing with pre-treatment of the biomass and taking away the inhibitors that are a by-product of fermentation

Microorganisms that ferment the glucose component in the cellulose to ethanol are well known in the art. However, the availability of microorganisms that efficiently ferment the 5-carbon sugar, xylose, in the hemicellulose to ethanol has been one of the bottlenecks in ethanol production from biomass.

Recently, Pretorius *et al.* (Food Technol. Biotechnol. 41:3-10, 2003) focused on the need of designing *Saccharomyces cerevisiae* for more efficient use of wood and wood products for ethanol production, i.e. the need for genetic modification of yeast. However, the genetically modified yeast strains described tend to be less efficient.

Patil *et al.* (Enzyme Microb. Technol., 1990, vol 12, 141-148) suggest the addition of fungal mycelium to accelerate ethanol production from cane molasses batch fermentation using Saccharomyces cerevisiae. The following fungi were investigated: *Penicillium chrysogenum, Aspergillus oryzae, Sclerotium rolfsii, Sporotrichum pulverluentum, Aspergillus niger, Rhizopus nigircans, Neurospora sitophilia, Fusarium tricinctum*, and *Trichoderma reesei.* The authors conclude that mycelium supplement with as many as 10 different fungal species could accelerate ethanol production, and advocate the use of waste mycelium from the antibiotic industry. Trace amounts of antibiotics present in the mycelium are believed to be beneficial in the removal of bacterial contamination during fermentation.

In light of the above, it remains to be developed alternative approaches to enhanced ethanol fermentation from organic starting materials, and in particular industrially applicable and economically competitive processes. One aim of the present invention is to make available such processes without resorting to genetic modification of the microorganisms involved.

Further aims underlying the invention, and advantages associated with the invention, will be evident to a skilled person from the description and examples.

Summary of the invention

The present invention makes available an improved process for the production of ethanol through fermentation of one or more organic starting materials, characterized by the features enumerated in the claims, incorporated herein by reference.

The invention also makes available a starter medium, as defined in the claims, incorporated herein by reference.

Further, the invention presents a growth medium for a fungus used in the inventive process.

Short description of the drawings

The invention will be described in closer detail in the following description, non-limiting examples, and attached drawings, in which:

Figure 1 shows the growth of *C. parvispora* for 65 h on xylose as the main carbon source in SH medium.

Figure 2 shows the growth of *C. parvispora* for 69.5 h on mannose as the main carbon source in SH medium.

Figure 3 shows the growth of *C. parvispora* for 66 h on galactose as the main carbon source in SH medium.

Figure 4 shows the growth of *C. parvispora* for 50 h in starch.

Figure 5 shows the growth of *C. parvispora* for 115 h in an experimental hydrolysate.

Figure 6 shows the accumulated ethanol production in wood hydrolysate with different amounts of yeast and microorganisms. 1 = 0.05g *C. parvispora*, 0.02g *S. cerevisiae*; 2 = 0.025g *C. parvispora*, 0.01g *S. cerevisiae*; 3 = 0.2g *C. parvispora*, 0.08g *S. cerevisiae*; 4 = 0.05g *C. parvispora*, 0.04g *S. cerevisiae*; 5 = 0.10g *C. parvispora*, 0.02g *S. cerevisiae*.

Figure 7 shows the accumulated ethanol production in a newly designed hydrolysate with 0.2g *C. parvispora* and 0.08g *S. cerevisiae* (g fresh weight (FW)/l).

Figure 8 shows the ethanol production in a wood hydrolysate with 0.2g *C. parvispora*

Detailed description of the invention

The present invention relates to a process for enhanced production of ethanol from biomass. It is based on the surprising discovery of a group of microorganisms capable of fermenting 5-carbon, and even capable of fermenting both 5-carbon and 6-carbon sugars, as well as their utility in ethanol production.

More specifically, the present invention relates to a process for the production of ethanol through fermentation of organic starting materials, wherein at least one fungus capable of metabolizing 5-carbon compounds is used. Said at least one fungus is also capable of fermenting 6-carbon compounds. Said at least one fungus is preferably chosen from the species belonging to *Chalara* spp., a group of wood

inhabiting fungi. Most preferably said at least one fungus is *Chalara parvispora,* a species growing well on 5-carbon sugars as well as 6-carbon sugars.

The most frequently used microorganism in hexose fermentation is *S. cerevisiae*. *S. cerevisiae* can produce ethanol from glucose and mannose if the concentration of sugars are high or when the yeast is grown under anaerobic conditions. Thus, according to one embodiment of the present invention the fungus is used in combination with at least one type of yeast. The yeast may belong to a species of *Saccharomyces*, preferably *S. cerevisiae*. Other species of yeast that can be used are, for example, species belonging to *Candida sp.*, such as *C. sheteae,* species belonging to *Pichia sp.* such as *P. bovis,* and species belonging to *Clasvispora sp.*.

The fungus can also be used in combination with other ethanol producing microorganisms to optimize substrate utilization, both 5-carbon metabolizing microorganisms and/or 6-carbon metabolizing microorganisms. For example, there are strains of fungi (e.g. *Fusarium, Mucor, Monilia* and *Paecilomyces)* that are able to produce ethanol from D-xylose, but they are considered to produce less ethanol than yeast. Also, genetically modified microorganisms can be used.

Enzymes may also be added to the process in order to facilitate the degradation of substrates and to enhance ethanol production. For example, cellulase can be added to degrade cellulose and hemicellulase to degrade hemicellulose. There are numerous examples of additional enzymes that can be used to degrade substrates to enhance ethanol production, two such examples are aldose reductase and xylitol reductase to degrade xylose. Other means of facilitating the degradation of substrates can be used, such as mechanical disruption, ultrasonication, or steam and high-pressure pretreatments.

In the process according to the invention said at least one fungus and said yeast are multiplied separately before use in a bioreactor. The fungus can be added to the organic material prior to the yeast or substantially simultaneously with the addition of the yeast. When the yeast is *S. cerevisiae,* it is cultured for about 24 h before addition to the biomass. The *Chalara* cultures are grown for about 24 - 48h, i.e. until they reach log phase, before addition to the starting material. About 0.05 to 0.2g cells (fresh weight) are added per litre.

In process of the invention the pH of the starting materials is adjusted to the range of about pH 5 – 6.5, preferably 5.5 – 6.2, and most preferably about pH 6. The pH may be adjusted by the addition of appropriate amounts of an alkali or an acid according to well-known procedures. The fermentation is performed in a temperature interval of about 26 to about 29 °C, preferably 27 °C. Other fermentation conditions, such as agitation, addition of co-substrates, nutrients, time and degree of anaerobiosis can be optimized according to the nature of the starting material and the fermenting microorganism(s) used.

The process according to the invention can be performed as a batch fermentation, wherein the microorganisms are killed or otherwise discarded after the fermentation. In another embodiment of the invention the fermentation process is performed as a continuous or semi-continuous process, where starting materials and/or nutrients are added during fermentation. To retain the microorganisms in the bioreactor they can be separated from the solids by any suitable means, for example sedimentation or centrifugation.

To obtain the ethanol after the fermentation the biomass first need to be separated from the fluids by means such as centrifugation or sedimentation. Subsequently, the ethanol can be separated from the biomass by any conventional method, such as distillation, membrane separation, enzyme process and gasification.

According to the invention the starting material can be any organic material that can be fermented for the production of ethanol. The ethanol can be produced from any lignocellulosic biomass. Relevant starting material include wooden or non-wood plant material, e.g. stem, stalk, shrub, hulls, foliage, fibre, bark, shell, root, straw, hay, grass, reed etc. Sources of wood can be any species of coniferous, hardwood and broad-leaf trees. Sources of straw include in particular cereals and cereal grasses, such as oat, wheat, barley, rye, maize and rice. Additional sources can be root-crops.

Further example of starting materials include waste or by-products from forestry, such as wood chips, saw dust etc; as well as solid or liquid effluents or by-products from pulp and paper industry, such as wood hydrolysates in different degrading states; paper waste, such as newspapers, magazines, photocopying and computer printer papers and paper based packaging. Preferred starting materials include spent liquor or waste liquor from pulping, such as black liquor, black liquor from sulphate

pulp cook or soda pulp cook, acidic waste liquor, acidic sulphite waste liquor, neutralized waste liquor etc.

Further example of starting materials include solid or liquid effluents or by-products from food and feed industry, for example effluents or by-products containing cellulose, hemicellulose, sugar or starch; solid or liquid waste or by-products from agriculture; by-products from gardening such as garden refuse or other waste or by-product streams or their components comprising compounds that can be fermented.

The starting material may be any of the above-mentioned materials in treated or untreated from. A skilled person without inventive effort can implement possibly necessary pretreatment steps.

The present invention also relates to a starter culture for use in the inventive process. The starter culture comprises at least one fungus capable of metabolizing 5-carbon compounds. Preferably said at least one fungus is also capable of metabolizing 6-carbon compounds. In one embodiment the fungus is chosen from the fungi belonging to the species *Chalara*. Preferably said at least one fungus is *C. parvispora*. The starter culture may be used in combination with other microorganisms, such as other fungi, yeasts and bacteria.

The present invention also relates to a growth medium for a fungus used in the inventive process. The medium is based on the commercially available SH medium (Schenk and Hildebrandt medium) adapted to the requirements of the fungi of the present invention. The composition is given in Table 1 (the concentration given as approximate values):

Table 1. Growth medium

Component	**Final concentration (gram/litre)**
$CaCl_2\ 2H_2O$	0.0125
$MgSO_4\ 7H_2O$	0.025
K_2HPO_4	1.0
$NaH_2PO_4\ 2H_2O$	0.67
D-xylose	25
D-mannose	25
D-galactose	25
NH_4Cl	1

The growth medium of Table 1 may further comprise starch at a final concentration of about 25 g/l.

The present invention also relates to the use of at least one fungus belonging to the *Chalara* species for the fermentation of an organic starting material in the production of ethanol. Preferably said at least one fungus is *C. parvispora*. The starting material can be any of the above-mentioned starting materials. Said at least one fungus can also be used in combination with other microorganisms, such as fungi, yeasts and bacteria. Preferably the fungus is used in combination with a yeast belonging to the species *Saccharomyces,* such as *S. cerevisiae*.

According to another embodiment, the invention relates to the use of at least one fungus belonging to the *Chalara* species for the manufacture of a starter culture for the use in the production of ethanol. Said at least one fungus is preferably *C. parvispora*. The fungus can also be used in combination with other microorganisms, such as fungi, yeasts and bacteria. Preferably the fungus is used in combination with a yeast belonging to the species *Saccharomyces,* such as *S. cerevisiae*.

The present inventors have shown that ethanol production from biomass can be increased by as much as 400% compared to fermentation using only the well known *Sacharomyces cerevisiae* (baker's yeast). Thus, this invention is of high economic and environmental interest.

One important advantage of the invention is that the ethanol production can be optimized with only minor changes in existing processes, meaning e.g. that there is no expense for rebuilding existing bioreactors. Consequently, the cost for ethanol production can be significantly reduced in existing bioreactors. If the cost for ethanol production is reduced, the use of ethanol as a replacement for fossil fuels will be more attractive.

The present invention will now be described in the following non-limiting examples.

Examples

Example 1. Growth of *Chalara parvispora* in growth medium supplemented with different carbon sources

Materials & Methods

In each experiment fifteen 100 ml bottles were used. The cultures were inoculated with 0.05 g FW fungi/l growth medium (see above) and grown at 27°C for 50 to 65h (se below) and were randomly weighed (wet weight), three bottles at four or five different times (in addition to time zero).

The growth of *C. parvispora* on different carbon sources was investigated by supplementing the medium with xylose 25 g/l, mannose 25 g/l, galactose 25 g/l and starch 25 g/l. respectivley. The growth of *C. parvispora* in a newly designed hydrolysate was also investigated and the growth recorded as described above. The cultures supplemented with xylose were weighed 17, 24, 41, 48, and 65 h after inoculation. The cultures supplemented with mannose were weighed 14.5, 38.5, 45.5, 62.5, and 69.5 h after inoculation. The cultures supplemented with galactose were weighed 18, 24, 43, 48, and 66 h after inoculation. The cultures supplemented with starch were weighed 17, 24, 36 and 50 h after inoculation. The cultures grown in wood hydrolysate were weighed 19, 43, 67, 91, and 115 h after inoculation.

Results

The results are summarized in the diagrams attached as Figures 1 through 5. The diagrams in Figures 1 through 4 show that *C. parvispora* grows equally well on xylose, mannose, galactose and starch as the carbon source, respectively. It is thus shown that *C. parvispora* is able to ferment both 5-carbon and 6-carbon compounds.

C. parvispora is also able to grow in a wood hydrolysate. Growth of *C. parvispora* in a hydrolysate showed an even better growth (Figure 5) than that registered for any single carbon source.

Example 2. Ethanol production in wood hydrolysate

Ethanol production in wood hydrolysate was investigated with different amounts of yeast (*S. cerevisiae*) and *C. parvispora* (see Table 2).

Materials & Methods

The yeast *S. cerevisiae* and the fungus *C. parvispora* were grown separately in YEP- and SH-medium for 24 and 48 h, respectively. YEP is a medium based on YPD, a complex medium for routine growth, but is without dextrose and can be used as a base for making media with alternate carbon source. At the start of the ethanol production experiments, different amounts of the microorganisms (see Table 2) were introduced into 100 ml flasks containing a wood hydrolysate (pH set to 6.0). The flasks were argonized to obtain an anaerobic atmosphere and subsequently incubated at 27°C for 113h under agitation (150 rpm/h).

Table 2. Amount of microorganisms used for production of ethanol in wood hydrolysate

Sample	Amount of *S. cerevisiae* (g)	Amount of *C. parvispora* (g)
1	0.02	0.05
2	0.01	0.025
3	0.08	0.2
4	0.04	0.05
5	0.05	0.10

Results

The result can be seen in Figure 6. The amount of ethanol produced was highest in sample 3 inoculated with concentration of *S. cerevisiae* of 0.08 g/l and a concentration of *C. parvispora* of 0.2 g/l. It is obvious that with a higher amount of *C. parvispora* more 5-C compounds are digested from the wood hydrolysate and are made available for the *S. cerevisiae* to utilize as a substrate for ethanol production. However, the yeast was not grown in pulp waste before start of the experiment.

Example 3. Ethanol production from lignocellulose in an experiemental hydrolysate

In this experiment ethanol production using *S. cerevisiae* and *C. parvispora* in an experimental hydrolysate was investigated.

Materials & Methods

Three bottles with 100 ml of an experimental hydrolysate (See Table 3), containing *S. cerevisiae* and *C. parvispora* , was argonized to anaerobiosis. Samples of accumulated ethanol production was taken after 19, 43, 66, 91 and 137h and analyzed by gas chromatography.

Table 3. Components of the experimental hydrolysate

Xylose	11 g/l
Mannose	27 g/l
Glucose	9.7 g/l
Galactose	4.7 g/l
Arabinose	0.69 g/l
Salts	0.0375 g/l
Phosphate buffer	1.67 g/l
NH_4Cl	1 g/l
Sterilized water up to 1 l	

Results

The results are shown in Fig. 7. A clear increase in ethanol production was observed as compared to the results shown in Fig. 6, i.e. about 17 g ethanol/l compared to 6.8 g ethanol/l. The increase is believed to be due to the fact that less inhibitory substances are present in the medium, which contains only pure chemicals.

Example 4. Ethanol production from lignocellulose in pulp waste

In this experiment ethanol production from lignocellulose in pulp waste was investigated. Ethanol production in pulp waste with *S. cerevisiae* was compared to ethanol production from pulp waste with both *S. cerevisiae* and *C. parvispora*.

Materials & Methods

Ten bottles each containing 100 ml of pulp waste (obtained from a pulp and paper mill) was used. Before the start of the experiment the *C. parvispora* was grown in wood hydrolysate for 24h, in order for the fungi to adapt to the pulp waste. Three bottles were inoculated with *S. cerevisiae* only and 3 bottles were inoculated with both *S. cerevisiae* and *C. parvispora*. The four remaining bottles were used as controls and contained YEP-medium and both the microorganisms. All bottles were put under anaerobic atmosphere by flushing with argon and thereafter kept shaking (15-20 rpm/min) at 27°C. The amount of produced ethanol was measured after 164.8 h using gas chromatography

Results

The results are shown in Table 4. The amount of ethanol produced from *S. cerevisiae* alone in pulp waste was 5.84 g/l and from *S. cerevisiae* and *C. parvispora* in combination in pulp waste was 23.43 g/l. Thus, there was a nearly 4-fold increase in ethanol production by the addition of *C. parvispora*. This experiment showed that ethanol production in lignocellulose waste from the pulp industry can be increased from 5.84 to 23.43 g/l, i.e. an increase by about 400%, by the use of an additional microorganism, i.e. *C. parvispora*. In addition, the results show that the *C. parvispora* can be "trained" to tolerate the pulp waste since the ethanol production in this experiment was higher than in the designed hydrolysate. The production can probably be further improved by the use of agents adsorbing the rest products of phenols and extractives.

Table 4. Amount of ethanol produced from lignocellulose in pulp waste.

	Amount ethanol produced (g/l)
S. cerevisiae + pulp waste	5.84
S. cerevisiae + *C. parvispora* + YEP medium	18.68
S. cerevisiae + *C. parvispora* + pulp waste	23.43

Example 5. Ethanol production from *C. parvispora*

Different *C. parvispora* strains were grown in SH-medium for 48 h. At the start of the ethanol production experiments, 0.2 g FW of the microorganisms was introduced into 10 ml tubes containing wood hydrolysate (pH 6.0). The tubes were argonized to obtain anaerobic atmosphere and thereafter kept at a constant temperature of 27°C and agitated (150 rpm/h). The experiment was run for 118h.

Results

The results as shown in Figure 8 clearly show that *C. parvispora* strains 983 and 385 as well as a new proprietary isolate (characterization not completed yet) have the capability of producing ethanol in a wood hydrolysate (WH).

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

Claims

1. A process for the production of ethanol through fermentation of organic starting material, **characterized** in that at least one fungus belonging to the species *Chalara* is used, said fungus being capable of metabolizing 5-carbon compounds.

2. The process according to claim 1, wherein said at least one fungus is capable of metabolizing both 5-carbon and 6-carbon compounds.

3. The process according to claim 1, wherein said at least one fungus is added to the organic starting material prior to, or substantially simultaneously with the addition of the yeast.

4. The process according to claim 1, wherein said fungus is used in combination with at least one yeast.

5. The process according to claim 1, wherein said at least one fungus is *Chalara parvispora*.

6. The process according to claim 1, wherein said yeast is a yeast belonging to the species *Saccharomyces*.

7. The process according to claim 6, wherein said yeast is *Saccharomyces cerevisiae*.

8. The process according to claim 1, wherein the fermentation is performed as a batch fermentation.

9. The process according to claim 1, wherein the fermentation is performed as a continuous or semi-continuous process, where starting materials and/or nutrients are added during fermentation.

10. The process according to claim1, wherein the pH of the starting material is adjusted to the range of about pH 5 – 7, preferably 5.5 – 6.5, and most preferably about pH 6.

11. The process according to claim 1, wherein the fermentation is performed in a temperature interval of about 26 to about 29 °C.

12. The process according to claim 1, wherein the starting material is chosen among:

- wood or non-wood plant materials;
- waste or by-products from forestry, such as wood chips, saw dust etc;
- solid or liquid effluents or by-products from pulp and paper industry, such as wood hydrolysates
- solid or liquid effluents or by-products from food and feed industry, for example, effluents or by-products containing cellulose, hemicellulose, sugar or starch;
- solid or liquid waste or by-products from agriculture;
- other waste or by-product streams or their components comprising compounds that can be fermented to produce ethanol; and
- any of the above mentioned materials in treated or untreated from.

13. A process for the production of ethanol from a starting material consisting substantially of waste or by-products from forestry, **characterized** in that at least one fungus belonging to the species *Chalara* is used, said fungus being capable of metabolizing 5-carbon compounds.

14. A process for the production of ethanol from a starting material consisting substantially of waste or by-products from forestry, **characterized** in that a combination of at least one yeast and at least one fungus belonging to the species *Chalara* is used, said fungus being capable of metabolizing 5-carbon compounds.

15. The process according to claim 13 or 14, wherein the starting material comprises spent liquor (waste liquor) from pulping.

16. A starter culture for use in a process according to claim 1, 13,14, or 15, comprising at least one fungus belonging to the species *Chalara*, said fungus being capable of metabolizing 5-carbon compounds.

17. The starter culture according to claim 16, wherein said at least one fungus is capable of metabolizing both 5-carbon and 6-carbon compounds.

18. The starter culture according to claim 16, wherein said at least one fungus is chosen from the fungi belonging to the species *Chalara parvispora*.

19. The starter culture according to claim 16, further comprising a yeast.

20. A growth medium for a fungus used in the process according to claim 1, 13,14, or 15, comprising $CaCl_2$ $2H_2O$ at a final concentration of about 0.0125 g/l, $MgSO_4$ $7H_2O$ at a final concentration of about 0.025 g/l, K_2HPO_4 at a final concentration of about 1.0 g/l, NaH_2PO_4 $2H_2O$ at a final concentration of about 0.67 g/l, D-xylose at a final concentration of about 25 g/l, D-mannose at a final concentration of about 25 g/l, D-galactose at a final concentration of about 25 g/l, and NH_4Cl at a final concentration about 1 g/l.

21. The growth medium according to claim 20, further comprising starch at a final concentration of about 25 g/l.

Abstract

Ethanol production from biomass can be rendered more effective by the use of a fungus capable of fermenting 5-carbon sugars, or both 5-carbon as well as 6-carbon sugars. Preferably said fungus is used in combination with other fermenting microorganisms, such as *Saccaromyces cerevisiae*.

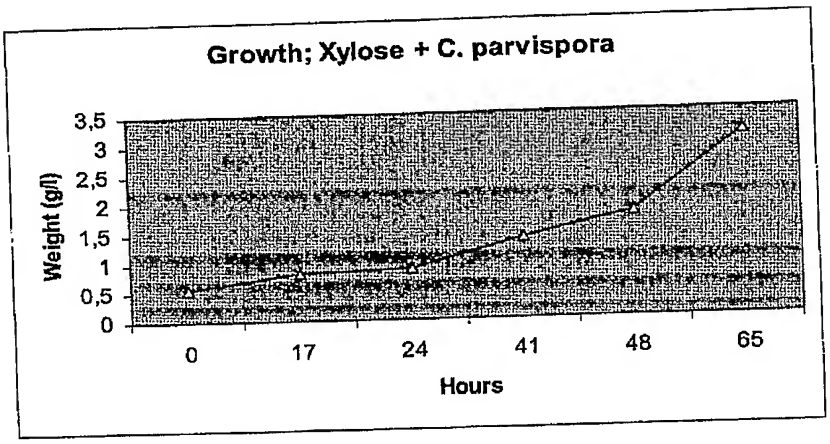

FIG. 1

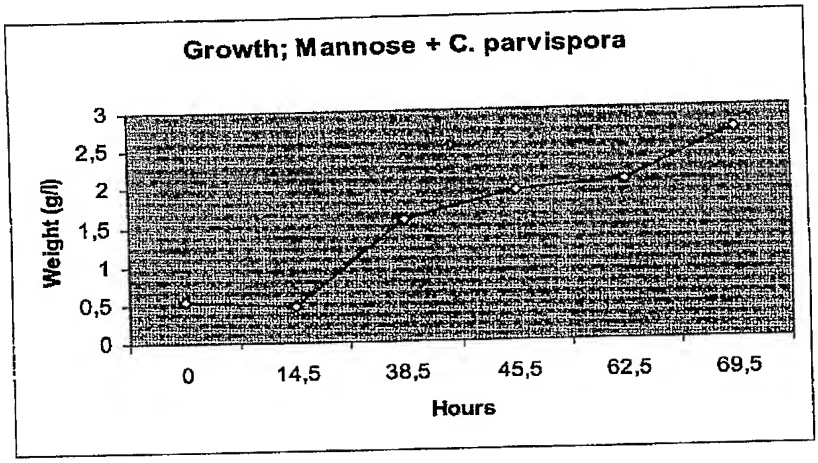

FIG. 2

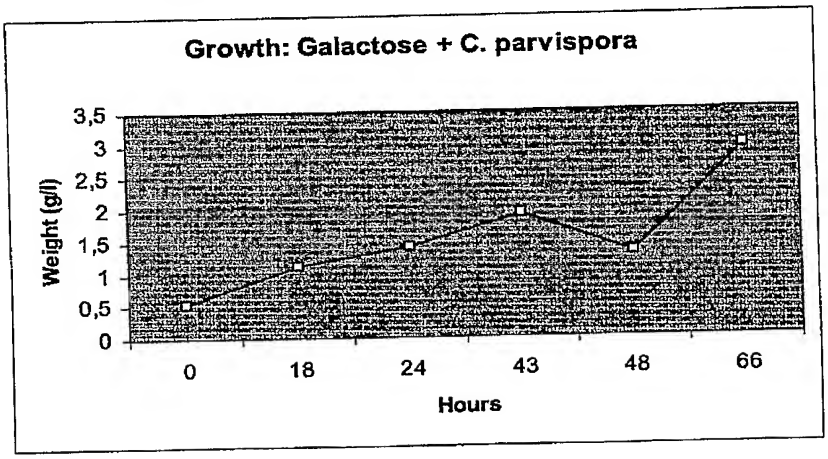

FIG. 3.

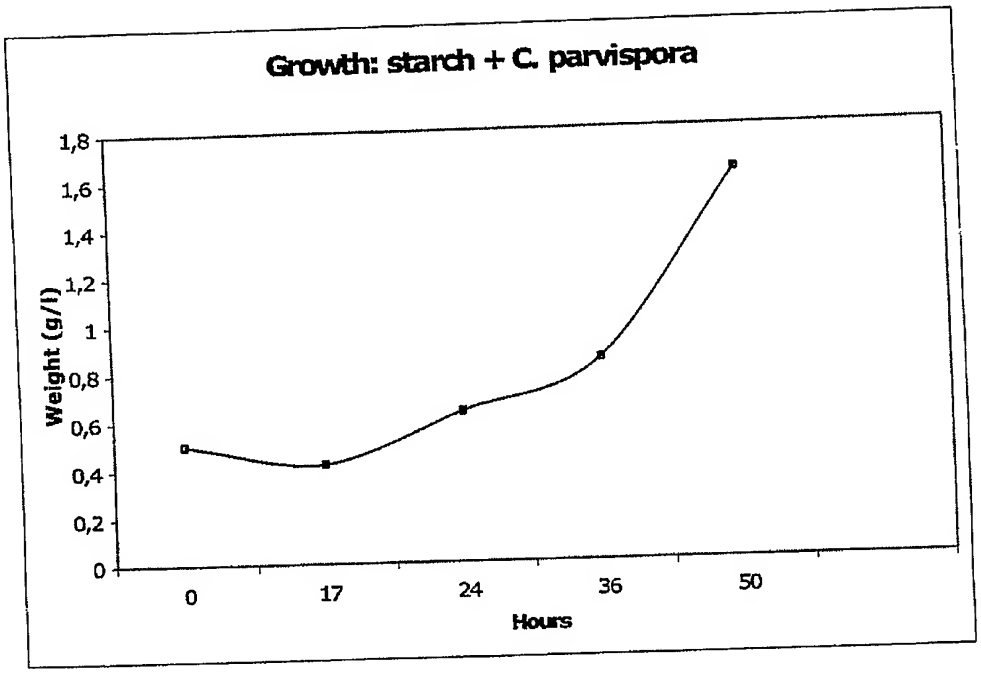
Growth: starch + C. parvispora
Weight (g/l)
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
17
24
36
50
Hours

FIG. 4

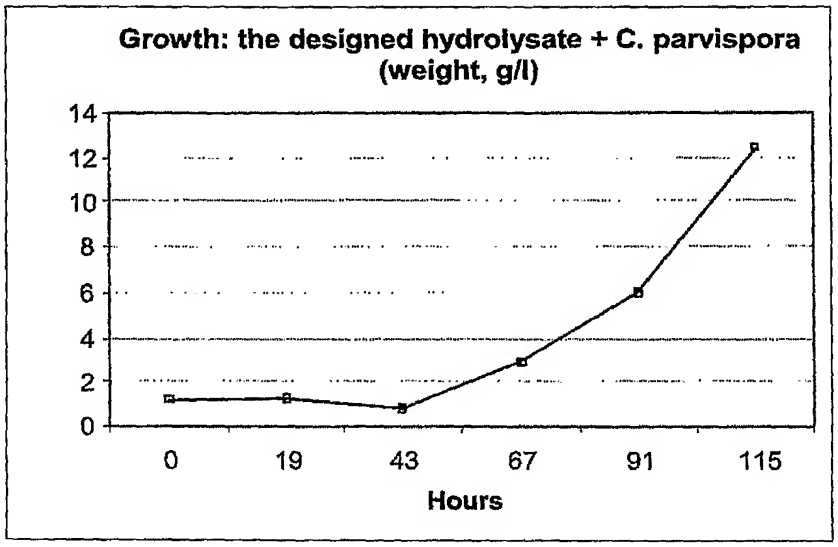
Growth: the designed hydrolysate + C. parvispora (weight, g/l)
14
12
10
8
6
4
2
0
0
19
43
67
91
115
Hours

FIG. 5

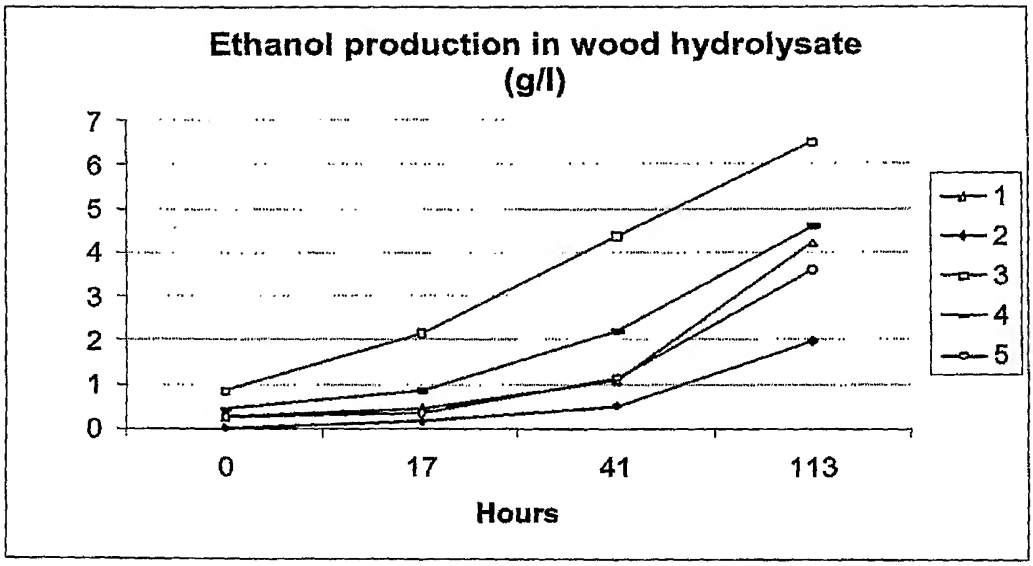
Ethanol production in wood hydrolysate (g/l)
7
6
5
4
3
2
1
0
0
17
41
113
Hours
1
2
3
4
5

FIG. 6

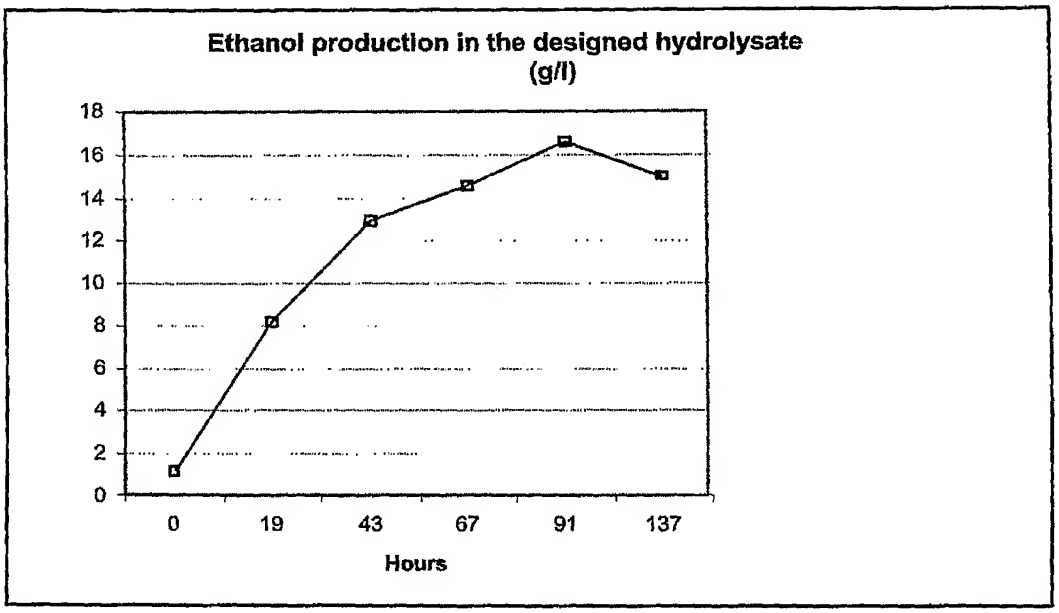
Ethanol production in the designed hydrolysate (g/l)
18
16
14
12
10
8
6
4
2
0
0
19
43
67
91
137
Hours

FIG. 7

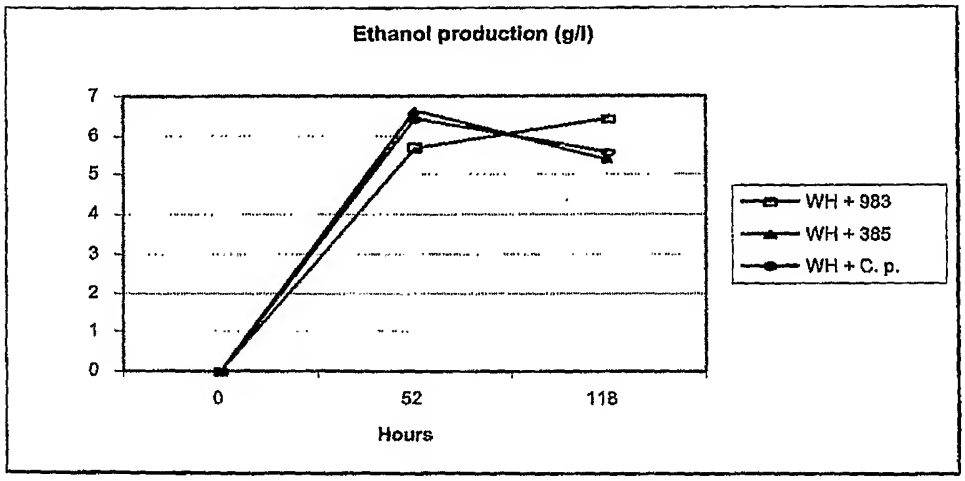
Ethanol production (g/l)
7
6
5
4
3
2
1
0
0
52
118
Hours
WH + 983
WH + 385
WH + C. p.

FIG. 8